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Destruction by Anaerobic Mesophilic and Thermophilic Digestion of Viruses and Indicator Bacteria Indigenous to Domestic Sludges

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16. ABSTRACT

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Destruction by Anaerobic Mesophilic and Thermophilic Digestion of Viruses and Indicator Bacteria Indigenous to Domestic Sludges

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In raw sludges and in mesophilically and thermophilically digested anaerobic sludges, large variations in numbers of viruses occurred over narrow ranges of numbers of fecal coliforms, total coliforms, and fecal streptococci, demonstrating that the bacteria were poor quantitative reflectors of the numbers of the viruses detected. Mesophilic and thermophilic digestion of anaerobic sludges destroyed all three indicator bacteria more rapidly than such digestion destroyed the viruses. The relative rates for the destruction of viruses, fecal coliforms, and fecal streptococci in the digested sludges were consistent over the 17-month study. Fecal coliforms were 7 to 8 times more sensitive than the viruses to mesophilic digestion and 9 to 10 times more sensitive to thermophilic digestion. Total coliforms were even more sensitive. The rates at which fecal streptococci were destroyed by mesophilic and thermophilic digestion of anaerobic sludges approached those at which the viruses were destroyed by those processes; this suggested that the rates at which fecal streptococci in sludges are destroyed by those processes may serve as useful indicators for the rates at which viruses in sludges are destroyed by those processes.

The 1972 Amendments to the Water Pollution Control Act (7) directed that all sewage treatment plants in the United States must establish secondary treatment facilities and thereby mandated the production of large quantities of virus- and bacteria-bearing sludges, most of them destined for discharge to the environment. Inasmuch as these sludges are generated within sewage treatment plants, it is possible at one point in time and at one place to disinfect them and thereby make them microbiologically safe for discharge. Because mesophilic digestion of anaerobic sludges (~35°C) has long been used to stabilize sludges and thereby make their odors aesthetically acceptable for discharge to the environment, the extent to which viruses and indicator bacteria are destroyed by this process is of obvious importance. Moreover, extended thermophilic digestion (~49°C) is an alternative to mesophilic digestion, and the virus-destroying and indicator bacteria-destroying capabilities of this high-temperature sludge stabilization process are also of great interest.

The study reported herein demonstrates the usefulness of fecal coliforms, total coliforms, and fecal streptococci as indicators of viruses in raw and digested sludges and the extent to which indicator bacteria and some viruses are destroyed by anaerobic mesophilic and thermophilic digestion of domestic wastewater sludges as these sludge stabilization processes are currently practiced.

(This study was part of a broad investigation of the inactivation of pathogens by sludge stabilization techniques. The coordinator for the study was Gerald Stern, Ultimate Disposal Section, Treatment Process Development Branch, Wastewater Research Division, Municipal Environmental Research Laboratory-Cincinnati, U.S. Environmental Protection Agency. Other parts of the investigation have been reported elsewhere [8].)

MATERIALS AND METHODS

Sludges. The sludges used in this study were obtained from the City of Los Angeles Hyperion Treatment Plant. The raw sludges were mixtures of primary sludge (two-thirds) and activated sludge (one-third). Anaerobic mesophilically digested sludges were obtained from digesters operating at ~35°C, and anaerobic thermophilically digested sludges were obtained from digesters operating at ~49°C. Each digester had a capacity of almost 9×10^6 liters (2.5×10^6 gallons). The digesters were fed and drawn down daily; the feeding always preceded the draw. Samples of digested sludges were obtained from the draw. The calculated average residence time of the digested sludges was 20 days.

Transport of sludges. Sludges destined for viral analyses were frozen in Dry Ice, transported to the laboratory, and maintained in a freezer at −70°C until

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extracted and assayed. In one series of studies, however (series 2), a duplicate set of samples was transported to the laboratory on wet ice. Because there was little difference in the numbers of viruses recovered from iced and frozen sludges, the data obtained with the two transport methods were averaged. Sludges destined for bacterial analyses were transported on wet ice to the laboratory and were assayed within 25 h after collection.

Recovery of viruses from sludges. Frozen sludges were thawed in cool water. Thawed sludges were mixed, and 2.66 ml of 10% beef extract (Oxoid beef extract powder) in McIlvaine buffer (0.05 M Na_2HPO_4 and sufficient citric acid to yield a pH of 7) was added per g of sludge. The sludges and beef extract were mixed together on a magnetic stirrer for 30 min and centrifuged at 10,000 × *g* for 30 min. The sediments were discarded. To remove bacteria and fungi, the supernatant was filtered through a 0.45-μm Millipore membrane filter in a Swinnex syringe apparatus. The supernatant was subsequently assayed for viruses (manuscript in preparation).

Assays for viruses. Viruses were assayed by the plaque technique on BGM cells. The viral assay procedures have been described elsewhere (4). Essentially identical techniques were used for recovering viruses from raw, mesophilic, and thermophilic sludges.

Assays for indicator bacteria. Membrane filter assays for fecal coliforms, total coliforms, and fecal streptococci were done by standard methods (1).

RESULTS

Except for an occasional aberration, the numbers of viruses recovered from raw sludges and from anaerobic sludges digested mesophilically and thermophilically did not vary greatly over short spans of time. This was shown by the relatively small variations in the numbers of viruses usually recovered from sludges within a series (Table 1). (Each series spanned from 35 to 56 days. A total of 4.5 months separated series 1 from series 2; 8 months separated series 2 from series 3; 14.5 months separated series 1 from series 3.) The numbers of viruses recovered from sludges were lowest in samples collected during the spring months (series 1) and highest in the samples collected during the late summer and autumn months (series 2 and 3). The largest number of viruses was recovered from a sample (test 9) taken in the month of August, the only summer sample collected during the study. These variations in the numbers of viruses recovered are consistent with the known seasonal patterns of excretion for those enteroviruses that are usually recovered in BGM cells and, consequently, with the occurrence of those viruses in sewage (2). Except for an occasional aberration, relatively little variation in numbers of fecal coliforms, total coliforms, and fecal streptococci occurred in raw sludges within any series (short time spans) or even from series to series (long time spans).

Ratios of indicator bacteria to viruses. The range in ratios of indicator bacteria to viruses in the raw sludges exceeded two orders of magnitude. The range in ratios of indicator bacteria to viruses in anaerobic sludges digested mesophilically and thermophilically usually extended from one to two orders of magnitude (see Tables 2, 3, and 4).

The ratios of indicator bacteria to viruses were reduced considerably by mesophilic digestion of anaerobic sludges and were reduced even more by thermophilic digestion. The arithmetic mean values for the ratios of indicator bacteria to viruses were useful only for rough approximations, but they demonstrated some important phenomena. The arithmetic mean value for the ratio of fecal coliforms to viruses showed that the average ratio of these indicator bacteria to viruses in the raw sludges was reduced by 5- to 6-fold by mesophilic digestion of those sludges and by more than 30-fold by thermophilic digestion (Table 2). A similar mean ratio of total coliforms to viruses was reduced about 9-fold by mesophilic digestion of raw sludges and about 200-fold by thermophilic digestion (Table 3). The fecal streptococci seemed by far the indicator bacteria most stable to mesophilic and thermophilic anaerobic digestion. The mean ratio of fecal streptococci to viruses in raw sludges was reduced by only about 1.4-fold by mesophilic digestion and by only about 2.7-fold by thermophilic digestion (Table 4).

The usefulness of these bacteria as quantitative indicators for viruses in raw sludges is indicated by plots of the numbers of viruses against the numbers of fecal coliforms, total coliforms, and fecal streptococci in each raw sludge sample (Fig. 1). The relationship in the raw sludge between the numbers of viruses and the numbers of each indicator organism is clearly not quantitative. Although the number of each indicator organism in a sample of raw sludge always exceeded the number of viruses by several orders of magnitude, the total range for the numbers of viruses was about twice as great as the total range for the numbers of each indicator organism. The best line that could be drawn through a set of data points that related the number of viruses and the number of an indicator organism in a raw sludge was vertical or near-vertical, indicating that any count of indicator bacteria encountered could reasonably reflect any of a wide range of viral numbers. Since the numbers of viruses and indicator bacteria in raw sludges are the bases for the numbers of viruses and indicator bacteria in digested sludges, the quan-

TABLE 1. *Recoveries of viruses and indicator bacteria from raw, mesophilic, and thermophilic sludges*

Series no.	Test no.	Viruses (PFU[a]/100 ml)			Fecal coliforms (CFU[b]/100 ml)			Total coliforms (CFU/100 ml)			Fecal streptococci (CFU/100 ml)		
		Raw	Mesophilic	Thermophilic	Raw	Mesophilic	Thermophilic	Raw	Mesophilic	Thermophilic	Raw	Mesophilic	Thermophilic
1	1	380	30	<4	3.7×10^{8}	7.7×10^{6}	9.5×10^{4}	4.5×10^{9}	6.4×10^{7}	4.3×10^{1}	3.6×10^{7}	2.8×10^{6}	4.3×10^{1}
	2	550	50	<3.1	4.3×10^{8}	8.6×10^{6}	$<7.0 \times 10^{2}$	7.2×10^{9}	6.8×10^{7}	2.0×10^{4}	3.0×10^{7}	1.7×10^{6}	2.3×10^{1}
	3	1,530	—[c]	<2.8	5.0×10^{6}	—	5.3×10^{3}	1.5×10^{8}	—	1.3×10^{4}	1.3×10^{6}	—	3.0×10^{1}
	4	940	40	<2.7	4.0×10^{8}	3.7×10^{6}	$<3.0 \times 10^{2}$	9.6×10^{9}	4.8×10^{7}	$<6.7 \times 10^{2}$	5.6×10^{7}	1.5×10^{6}	$<6.7 \times 10^{2}$
2	5	2,780	280	3.3	4.8×10^{8}	1.1×10^{7}	4.7×10^{4}	4.8×10^{9}	6.3×10^{7}	1.4×10^{5}	3.2×10^{7}	2.2×10^{6}	4.7×10^{4}
	6	1,550	210	<1.4	3.4×10^{8}	4.1×10^{6}	8.2×10^{4}	7.9×10^{9}	6.3×10^{7}	2.3×10^{5}	4.1×10^{7}	3.0×10^{6}	3.5×10^{4}
	7	1,190	240	<1.7	6.4×10^{8}	6.4×10^{6}	4.0×10^{3}	1.0×10^{10}	7.4×10^{7}	9.6×10^{3}	2.5×10^{7}	2.4×10^{6}	2.9×10^{4}
	8	1,810	100	1.7	8.7×10^{8}	6.4×10^{6}	2.7×10^{4}	7.6×10^{9}	4.8×10^{7}	4.7×10^{4}	2.0×10^{7}	2.0×10^{6}	4.8×10^{4}
3	9	11,600	410	5.0	9.0×10^{7}	1.5×10^{6}	$<1.0 \times 10^{3}$	4.0×10^{8}	5.5×10^{7}	$<1.0 \times 10^{3}$	7.0×10^{6}	1.4×10^{6}	1.0×10^{1}
	10	2,470	200	4.6	3.0×10^{8}	6.0×10^{6}	7.0×10^{4}	1.2×10^{10}	1.3×10^{8}	2.9×10^{5}	5.0×10^{7}	2.1×10^{6}	6.0×10^{4}
	11	1,500	360	16.7	1.0×10^{8}	5.0×10^{6}	5.0×10^{4}	5.5×10^{9}	1.6×10^{8}	7.0×10^{4}	2.3×10^{7}	3.6×10^{6}	5.0×10^{4}

[a] PFU, Plaque-forming units.
[b] CFU, Colony-forming units.
[c] —, Not done.

TABLE 2. *Ratios of relative numbers of viruses and fecal coliforms in raw, mesophilic, and thermophilic sludges*

Series no.	Test no.	Fecal coliforms (CFU):viruses (PFU)					
		Raw sludge (A)	Mesophilic sludge (B)	Thermophilic sludge (C)	A:B	B:C	A:C
1	1	970,000:1	260,000:1		3.7:1		
	2	780,000:1	170,000:1		4.6:1		
	4	430,000:1	92,000:1		4.7:1		
2	5	170,000:1	39,000:1	14,000:1	4.4:1	2.8:1	12.1:1
	6	220,000:1	20,000:1		11.0:1		
	7	540,000:1	27,000:1		20.0:1		
	8	480,000:1	64,000:1	16,000:1	7.5:1	4.0:1	30.0:1
3	9	7,800:1	3,700:1		2.1:1	18.5:1	
	10	120,000:1	30,000:1	15,000:1	4.0:1	2.0:1	8.0:1
	11	67,000:1	14,000:1	3,000:1	4.8:1	4.7:1	22.3:1
Arithmetic mean		378,000:1	72,000:1	12,000:1			

TABLE 3. *Relative numbers of viruses and total coliforms in raw, mesophilic, and thermophilic sludges*

Series no.	Test no.	Total coliforms (CFU):viruses (PFU)					
		Raw sludge (A)	Mesophilic sludge (B)	Thermophilic sludge (C)	A:B	B:C	A:C
1	1	12,000,000:1	2,100,000:1		5.7:1		
	2	13,000,000:1	1,400,000:1		9.3:1		
	4	10,000,000:1	1,200,000:1		8.3:1		
2	5	1,700,000:1	220,000:1	42,000:1	7.7:1	5.2:1	40.5:1
	6	5,100,000:1	300,000:1		17.0:1		
	7	8,400,000:1	310,000:1		27.1:1		
	8	4,200,000:1	480,000:1	28,000:1	8.8:1	17.1:1	150.0:1
3	9	34,000:1	130,000:1		0.26:1		
	10	4,900,000:1	650,000:1	63,000:1	7.5:1	10.3:1	77.8:1
	11	3,700,000:1	440,000:1	4,200:1	8.4:1	104.8:1	880.9:1
Arithmetic mean		6,300,000:1	720,000:1	34,000:1			

titative relationships between them in digested sludges cannot be more quantitative than in raw sludges. Although not shown, plots of these relationships in digested sludges were similar to those in raw sludges.

Destruction of viruses and indicator bacteria by mesophilic and thermophilic digestion of anaerobic sludges. The relative usefulness of fecal coliforms, total coliforms, and fecal streptococci as indicators of the rate of destruction of viruses in mesophilically or thermophilically digested anaerobic sludges was determined from plots of the destruction of viruses against the destruction of each indicator organism (see Fig. 2 and 3). Each point on the plots relates for 1 sampling day the fraction of the viruses destroyed and the corresponding fraction of an indicator organism destroyed. Both are given as percentage of destruction. The diagonal marker line that has been drawn in is the line upon which would fall all points for which the fractions of viruses and indicator organisms destroyed were equal. Points above the line represent a greater fractional destruction of viruses than of the indicator bacteria; points below the line represent a greater fractional destruction of indicator bacteria than of viruses.

(i) Mesophilic digestion. The relationship of the relative destruction of viruses and fecal coliforms in each mesophilically digested sludge sample is presented in Fig. 2. A line parallel to but considerably beneath the marker line (which

TABLE 4. *Relative numbers of viruses and fecal streptococci in raw, mesophilic, and thermophilic sludges*

Series no.	Test no.	Fecal streptococci (CFU):viruses (PFU)					
		Raw sludge (A)	Mesophilic sludge (B)	Thermophilic sludge (C)	A:B	B:C	A:C
1	1	95,000:1	93,000:1		1.0:1		
	2	55,000:1	34,000:1		1.6:1		
	4	60,000:1	37,000:1		1.6:1		
2	5	12,000:1	7,900:1	14,000:1	1.5:1	0.56:1	0.86:1
	6	26,000:1	14,000:1		1.9:1		
	7	21,000:1	10,000:1		2.1:1		
	8	11,000:1	20,000:1	28,000:1	0.55:1	0.71:1	
							0.39:1
3	9	603:1	3,400:1	200:1	0.18:1	17.0:1	3.0:1
	10	20,000:1	10,000:1	13,000:1	2.0:1	0.77:1	1.5:1
	11	15,000:1	10,000:1	3,000:1	1.5:1	3.3:1	5.0:1
Arithmetic mean		32,000:1	24,000:1	12,000:1			

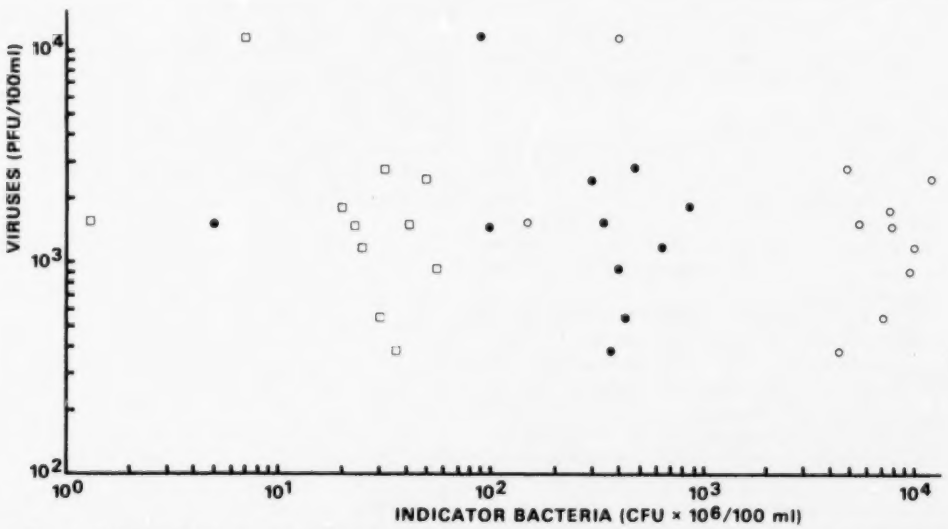

FIG. 1. *Relationship between numbers of indicator bacteria and numbers of viruses in raw sludges.* (●) *Viruses–fecal coliforms;* (○) *viruses–total coliforms;* (□) *viruses–fecal streptococci.*

represents a 1:1 relationship of indicator bacteria to viruses) may be drawn through the points which represent that relationship, especially if two of the points are excluded. Thus, in the mesophilic sludge digester, the numbers of fecal coliforms destroyed seem to have been generally proportional to the numbers of viruses destroyed. The numbers of fecal coliforms destroyed, however, were about seven to eight times greater than the numbers of viruses destroyed.

A similar line can be drawn to represent the relationship of the relative destruction of viruses and total coliforms, especially if three of the points are excluded. Such a line would fall beneath that representing the relationship between fecal coliforms and viruses and would indicate that in the mesophilic digester the destruction of total coliforms was about 9 to 10 times greater than the destruction of viruses. Fecal coliforms, of course, tolerate higher temperatures better than many of the bacteria in

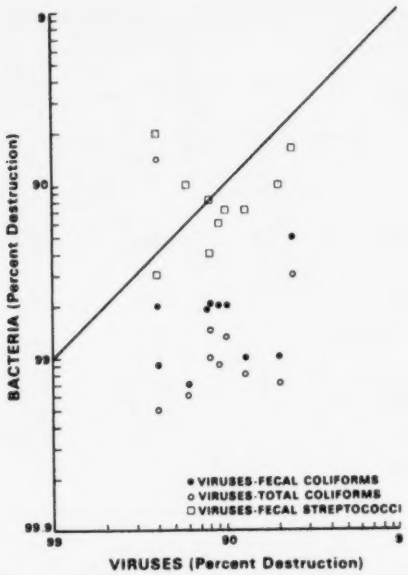

FIG. 2. *Relative destruction of viruses and indicator bacteria by mesophilic digestion of sludges.*

the total coliform group.

Most of the points that represent the relationship of the relative destruction of viruses and fecal streptococci fall just below the marker line. The number of fecal streptococci destroyed, therefore, reflects fairly closely the number of viruses destroyed by mesophilic digestion of anaerobic sludges.

(ii) Thermophilic digestion. Although a smaller number of data points attest to the findings, the numbers of fecal streptococci destroyed in thermophilically digested anaerobic sludges reflect relatively closely the numbers of viruses destroyed by the process (Fig. 3). The numbers of fecal coliforms destroyed in the thermophilic digester were consistently proportional to the numbers of viruses destroyed, but more than 10 times as many of the bacteria were destroyed as were viruses. The sensitivity of total coliforms to the conditions in the thermophilic digester, relative to that of the fecal coliforms, was considerably greater than to the conditions in the mesophilic digester. With only four data points, no pattern could be evolved for total coliforms in the thermophilic digester.

DISCUSSION

In intermittently fed digesters of the kind used for these studies, there is no direct way of determining how many viruses and bacteria are destroyed during digestion of anaerobic sludges. Raw sludge was fed daily to each digester, and each digester was drawn down shortly thereafter. Drawn sludges were therefore composite materials that had been digested for an average of 20 days. The number of viruses recovered from a raw feed sludge collected on the day on which digester sludges were sampled had to be used as the base for computing the numbers of viruses destroyed in the sludge digesters. The validity of this base depended upon the consistency of the numbers of viruses in the feed sludge over a period of time. The 11 tests described herein (Table 1) comprised three series of three or four tests each. Tests 1 to 4 (series 1) were done over a period of 35 days, tests 5 to 8 (series 2) were done over a period of 56 days, and tests 9 to 11 (series 3) were done over a period of 42 days. A period of 4.5 months separated the completion of the first series from the beginning of the second series, and a period of 8 months separated the completion of the second series from the beginning of the third series. The variations in the numbers of viruses recovered from raw sludges among tests within each series were usually small, suggesting that day-to-day variations in these numbers were relatively small. Moreover, differences in numbers of viruses and bacteria recovered from raw sludges even over the entire series of tests were small when compared to the reductions in numbers produced in the sludge digesters (Fig. 2 and 3). Thus, the

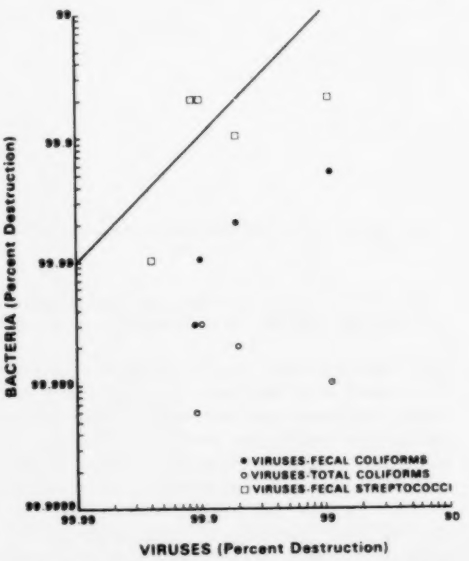

FIG. 3. *Relative destruction of viruses and indicator bacteria by thermophilic digestion of sludges.*

numbers of viruses in the sludges fed to the digesters on the day when digester sludges were assayed appear to have been reasonably valid reflections of the average virus feed, for the purpose of estimating the numbers of viruses destroyed in the digesters.

The data presented in Fig. 1 indicate that neither the fecal coliforms, the total coliforms, nor the fecal streptococci are good quantitative indicators for the presence of viruses in raw sludges. Neither can these bacteria be good quantitative indicators for the presence of viruses in mesophilically or thermophilically digested anaerobic sludges, since the relationships of indicator bacteria to viruses in the digested sludges cannot be better than they are in the raw sludges from which they originate. The numbers of viruses recovered from these sludges varied widely over a relatively narrow range in numbers of indicator bacteria recovered, demonstrating that either a relatively small or a relatively large number of viruses might be present at any given level of indicator numbers (2). However, many indicator bacteria always remained in samples in which viruses were no longer detected (Table 1). Thus, the smallest numbers of indicator bacteria present in samples from which viruses were not recovered may serve as a useful guidepost for adjudging sludges to be likely free of viruses—at least of those viruses that may be recovered in BGM cells.

It is clear that mesophilic and thermophilic digestion of anaerobic sludges reduces considerably the numbers of viruses and indicator bacteria in those sludges. In 20 days, under the conditions of the tests, mesophilic digestion reduced the numbers of viruses recovered from raw sludges by about 90% and reduced the numbers of fecal streptococci by something more than 90%. The numbers of fecal coliforms were reduced by about 98%, and the numbers of total coliforms were reduced by a little more than 99%. In 20 days, under similar test conditions, thermophilic digestion reduced the numbers of viruses recovered from raw sludges by 99 to 99.9% and reduced the numbers of fecal streptococci by a little more than 99.9%. The numbers of fecal coliforms were reduced by from 99.9 to >99.9999% by 20 days of thermophilic digestion, and the numbers of total coliforms were reduced to an even greater degree. Had samples for assay been obtained before digesters were fed rather than afterward, the numbers of viruses and indicator bacteria recovered would have been even smaller. Even so, viruses indigenous to raw sludges appear to be inactivated by digestion much more slowly than laboratory strains of viruses that are seeded into such sludges. Although mesophilic digestion reduced the numbers of viruses indigenous to raw sludges by about 90% in 20 days, laboratory strains of viruses seeded into sludges usually have been reduced in numbers by about 90% in 24 h (3, 5, 6, 9). Thus, data gathered with laboratory strains of seeded viruses appear to overestimate considerably the rate at which viruses indigenous to raw sludges are inactivated by mesophilic digestion.

Although the fecal streptococci were more sensitive than the viruses to both mesophilic and thermophilic digestion, the rate of destruction for these organisms seemed closely similar to the rate of destruction for the viruses recovered from sludges in these studies. Thus, fecal streptococci, in some instances at least, may serve as a useful indicator of fecal viruses. Fecal coliforms were destroyed in both mesophilic and thermophilic digesters at rates that were consistently higher than the rates at which the fecal streptococci were destroyed. Total coliforms were destroyed even more rapidly than fecal coliforms.

In any event, mesophilic digestion of anaerobic sludges for 20 days clearly reduces the hazard from viruses in sludges discharged to the environment in land or ocean disposal. Thermophilic digestion for the same period of time may destroy all of the viruses in such sludges. The common practice of feeding raw sludges to digesters before drawing down for discharge, however, probably adds viable viruses and indicator bacteria to the samples assayed and undoubtedly results in higher viral and bacterial counts than we would see if drawing down preceded feeding. Drawing down sludges before digesters are fed would undoubtedly produce a safer sludge for discharge than current common practice permits.

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